

CYTOLOGICAL ANALYSIS OF THE THELYTOKOUS PARTHENOGENESIS
IN THE CAPE HONEYBEE (Apis mellifera capensis ESCHOLTZ)

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CYTOLOGICAL ANALYSIS OF THE THELYTOKOUS PARTHENOGENESIS IN THE CAPE HONEYBEE(Apis mellifera capensis ESCHOLTZ).

The present study deals with the cytological examination of the automictic mechanisms of parthenogenesis in the developing oocytes and eggs of laying workers of Apis mellifera capensis bees. It is based on the cycles of meiosis, beginning with early prophase in the mature ovarian oocytes, progressing through two maturation divisions upto the onset of cleavage in the egg. The primary aim of the present work was to determine the cytological mechanism responsible for the stability of the chromosome number in the laying workers of the capensis bees.

In the honeybee(Apis mellifera L) eggs may develop either through fertilisation or without it. In the former case, the egg may develop into a diploid female and without fertilisation into a male. In certain races of honeybees, many research workers have reported the unusual occurrence of impaternate female among the male progeny of the virgin queens and laying workers, as in the South African cape bees(Onions, 1912, 1914), Jack(1916), Italian bees(Mackensen, 1943: Butler, 1954) and Caucasian bees(Mackensen 1943) etc.

Although occurrence of impaternate females in the above races of honeybee have been known for over 100 years, there has been a few investigations of the genetical mechanisms involved in the process and so far no cytological studies have been done in this regard. Genetic experiments by Mackensen(1943), Butler(1954) and Tryasko(1975) showed that impaternate females are diploid and further Tucker(1958), Kerr(unpublished data) and Ruttner(1977) suggested that such diploid workers are formed by automictic parthenogenesis. But there is no proof of the actual cytological mechanism involved in the process, which is being undertaken for the first time in the laying workers of the cape bees.

Among the different races of Apis mellifera L. the phenomenon of female parthenogenesis is the most remarkable in Apis mellifera capensis. Onions(1912) for first time reported that worker bees in the queenless colonies of capensis bees start developing ovaries and also possess spermatheca which is devoid of any kind of sperm cells. These laying workers of capensis bees produce a brood of the workers from which the queen may develop. These findings were later conformed by Jack(1916), Anderson(1963) and Ruttner(1976a). Ruttner(1976b) reviewed the taxonomy of the African bees and concluded that cape bees are characterized by the development of spermatheca and the presence of a large number of ovarioles in the ovaries of the laying worker bees. Like a real queen the laying worker bee is also surrounded by a 'court' of bees. They laid eggs exclusively in the worker cells of the comb, but the brood nest was scattered on the comb.

Pure genetic stock of Apis mellifera capensis supplied by the Institute für Bienkunde Frankfurt University, West Germany, was used for rearing the laying worker bees. First a productive method of producing a large number of impaternate females (laying workers) was evolved in order to obtain the eggs of the precise age on a mass scale for the cytological investigations. Young capensis bees which emerged from the brood were marked and introduced into small cages along with the young nurse bees of Apis mellifera carnica. About 4 to 3 capensis worker bees in each cage developed their ovaries in 7 to 10 days and immediately after that start laying eggs. These cages

were kept in an incubator at 26^{0}_{+10} . Eggs of a precise age were collected by marking the side and position of the egg in the comb cell as soon as it was deposited and allowing it to remain there for a desired period of time. The eggs were collected with a fine glass needle. A fine glass needle was prepared by stretching one of the end of a thin capillary tube and then the very thin end was blown to a bulb. The eggs attached easily to the bulb and could safely be removed from the comb cell.

The cytological methods employed to study the chromosomal behaviour and the process of automixis are the squash technique and the paraffin sectioning method (microtomy) respectively. For the squash preparations the eggs and the ovaries of the laying worker *capensis* bees were pre-treated with 0.55M Ohnuki's solution (1965). The material was fixed in acetic alcohol i.e. acetic and ethanol (1:3) for 20 to 30 minutes at room temperature and squashed with Aceto-carmine (Kitzmilller and Clark 1952) Aceto-orcein (Darlington and La Cour, 1976) and Lacto-Orcein (Sheppard, 1973). For the microtomy the eggs of the precise age (collected from fresh to six hours old with an interval of half an hour) were fixed in Petrunkwitsch's fixative (Petrunkwitsch, 1901). The sections were cut at 6.5 μ thick and stained with Heidenhein's iron haematoxylin procedure.

The cytological results showed that impaternate laying worker females of *capensis* bees are normal diploids with 32 as the chromosome number which also occurs in the queen and worker castes of *Apis cerana indica*, *Apis dorsata*, *Apis florea* and the different races of *Apis mellifera* (Fahernhorst, 1977). The first maturation division was initiated in the ovary before ovulation. During the first maturation division the chromosome pairing, synapsis, chiasmata and bivalents were observed during prophase diakinesis and metaphase I stages. These results show that an egg has undergone a process of reduction division and possesses haploid number of chromosomes ($n=16$) and it has also been confirmed from the number of the bivalents which are 16 in number in the diakinesis and metaphase I stages. The net result of the first meiotic division was the formation of two nuclei which lie parallel to the longitudinal axis of the egg or perpendicular to the surface of the egg.

During the second maturation division in anaphase II, two meiotic spindles were observed which also lie parallel to the longitudinal axis of the egg in one line. The position of the four nuclei formed as a result of the second maturation division corresponds with that of the queen egg (Petrunkwitsch, 1901; Nachtsheim, 1913). Based on his genetical experiments Tucker (1958) proposed a hypothetical scheme of the spindle misorientation in order to explain the nature of the fusion of the nuclei for the restoration of diploidy in the different races of *Apis mellifera*. Ordinarily, the first meiotic division anaphase spindle in the freshly laid eggs of the queen honeybee lies in a sagittal plane, parallel to the surface of the egg and then rotates through 90° , lying perpendicular to the surface of the egg (Petrunkwitsch, 1901; Nachtsheim, 1913).

According to Tucker (1958), the first meiotic spindle in the egg of laying worker fails to rotate and the first division is completed while the spindle is in the same position i.e. parallel to the surface of the egg. As a result of this at anaphase II and Metaphase II stages the two nuclei, facing the interior of the egg would unite, while the other two towards the periphery of the egg would degenerate.



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In this way ,two central nuclei from the different oocytes would unite.

However, in the present actual cytological observations the position of the anaphase I spindle of the egg of the laying worker is the same as that of the queen egg. The anaphase I stage spindle lies perpendicular to the surface of the egg but not as sagittal to the surface, as proposed by Tucker(1958). The second anaphase and second telophase stages also lie perpendicular to the surface of the egg. As a result of this all the four haploid nuclei are placed more or less in a single line. Out of these four nuclei ,the two central ones(non -sister nuclei, as they are derived from the two separate oocytes) which approximate each other ,finally unite to form the diploid zygote nucleus. The zygote nucleus which occupies the peripheral periplasmic area later on sinks deeper in to the interior of the egg and undergoes the cleavage division to form the future embryo.

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